UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

DATE: August 25, 2005

MEMORANDUM

TXR#: 0052528

SUBJECT: **Dicamba**: Reviews of 28-Day Dermal and 90-Day Oral Toxicity Studies

FROM: Yung G. Yang, Ph.D.
Toxicology Branch
Health Effects Division (7509C)

THROUGH: Alberto Protzel, Ph.D.
Branch Senior Scientist
Toxicology Branch
Health Effects Division (7509C)

TO: Kendra Tyler
Reregistration Branch
Registration Division (7508C)

DP Barcode: D301908/D249152 **Chemical:** Dicamba
PC Code: 029801 **CAS No.:** 1918-00-9

Action Requested: Review a 28-day dermal toxicity study and a 90-day oral toxicity study in rats on dicamba.

Response: The Toxicology Branch has reviewed the 28-day dermal toxicity study in rats (MRID 45814501) and the 90-day oral toxicity study in rats (MRID 44623101) and found that they are acceptable/guideline and may be used for regulatory purpose.

The Data Evaluation Records (DERs) are attached as follows.

ED_005172C_00001756-00001

Attachments
Data Evaluation Records
28-day dermal toxicity Study (MRID 45814501)
90-day oral toxicity study (MRID 44623101)

ED_005172C_00001756-00002

EPA Reviewer: Robert P. Zendzian, Ph.D.
Toxicology Branch], Health Effects Division (7509C)
EPA Secondary Reviewer: Yung G. Yang, Ph.D.
Toxicology Branch, Health Effects Division (7509C)

Signature: [signature]
Date 8/17/05
Signature: [signature]
Date 8/17/2005

Template version 11/01

TXR#: 0052528

DATA EVALUATION RECORD

STUDY TYPE: 28-Day Dermal Toxicity - [rat] ; OPPTS 870.3200 [§82-2]; OECD 410.

PC CODE: 029801 **DP BARCODE:** 301908.

TEST MATERIAL (PURITY): Dicamba (91.0 %)

SYNONYMS: 3,6-Dichloro-2-methoxylbenzoic acid

CITATION: Rattrey, N.J. (2002) Dicamba Tech (SAN 837 Tech): 28 day dermal toxicity study in rats. Syngenta Limited, Central Toxicology Laboratory, Alderly Park Macclesfield, Cheshire, UK. BASF Registration Document Number, 2002/5004466, October 29, 2002. MRID 45814501, unpublished

SPONSOR: BASF Corporation

EXECUTIVE SUMMARY:

In a 28-day dermal toxicity study (MRID 45814501), Dicamba (91.0% a.i., batch #B2826511) was applied to the shaved skin of 10 male and 10 female Alpk:AP ,SD rats /sex/dose at dose levels of 0, 30, 300 or 1000 mg/kg bw/day, 6 hours/day for 5 days/week during a 28-day period.

Clinical observations, body weights and food consumption were measured throughout the study. Urine samples were taken for clinical pathology during week 4 of the study. A functional observational battery of all animals consisting of: detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, and assessment of motor activity was performed on day 22. At the end of the scheduled period, the animals were killed and subjected to a post mortem examination. Blood samples were taken for clinical pathology, selected organs and specified tissues were taken for subsequent histopathological examination.

There were no changes indicative of systemic toxicity in either sex. There were no compound related effects in mortality, clinical signs, body weight, food consumption, hematology, clinical chemistry, organ weights, or gross and histologic pathology. Histopathological changes indicative of irritation were seen in skin from the application site in both sexes given 1000 or 300 mg/kg/day and in some males given 30 mg/kg/day.

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A LOAEL for systemic toxicity was not established. The NOAEL is 1000 mg/kg/day the highest dose tested.

This 28-day dermal toxicity study in the rat is acceptable/ guideline, and satisfies the guideline requirement for a 28-day dermal toxicity study (OPPTS 870.3200 ; OECD 410) in the rat.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided. No deviations from regulatory requirements were reported.

ED_005172C_00001756-00004

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Material:	Dicamba
Description:	Tech (SAN 837 tech) milled sample
Batch #:	B2826511
Purity:	91.0% a.i.
Compound Stability:	ambient temperature in dark
Source	Syngenta crop protection munchwilen AG
Color	white
CLR reference number	Y01040/007
Physical staste	solid

2. Vehicle and/or positive control: deionized water

3. Test animals:

Species:	rat	
Strain:	Alpk:AP ,SD(Wister derived)	
Age/weight at study initiation:	8 weeks old, 200-300 g body weight	
Source:	Rodent breeding unit, Alderly Park Macclesfield, Cheshire, UK	
Housing:	5 per sex per cage	
Diet:	RM1 Special Diet. Services Limited *ad libitum*	
Water:	*ad libitum*	
Environmental conditions:	**Temperature:**	22±3°C
	Humidity:	30-70 %
	Air changes:	15/hr
	Photoperiod:	12hrs dark/ 12hrs light
Acclimation period:	5 days in the laboratory	

B. STUDY DESIGN:

1. In life dates - Start: April 23, 2002 End: May 22, 2002

2. Animal assignment: Animals were assigned randomly to the test groups noted in Table 1.

ED_005172C_00001756-00005

TABLE 1: Study design.

Test Group	Dose (mg/kg bw/day)	# Male	# Female
Control	0	10	10
Low	30	10	10
Mid	300	10	10
High	100	10	10

3. Dose selection rationale

The dose levels selected for this study were based on the resultes of a preliminary dermal study in the rat carried out in the Laboratory (CTL Study Number LR0595).

4. Preparation, treatment of animal skin and dose administration

Two days before the first dermal application, the hair was removed from the dorso-lumbar region of each animal using veterinary clippers. Sufficient area was clipped to enable application of the foil backed gauze patch. Only animals in which the epidermis appeared to be intact and normal on gross observation immediately after clipping were used.

The amount of the dose was calculated for each animal according to its body weight at the time of dosing. The appropriate amount of test substance (allowing the following levels of variation: Group 2 $\pm$ 0.001 g, Groups 3 and 4 $\pm$ 0.003g) was weighed in a plastic weighing boat and mixed with the minimum quantity of de-ionized water to produce a paste to allow good skin contact. This was applied to the foil backed gauze patch, designed to cover approximately 10% of the surface area of the rat. The foil backed gauze patch was held in position by a cohesive bandage and 'blenderm' tape,

At the end of the six hour contact period, each dressing was removed by cutting through the tape and bandage and gently peeling the dressing off, The skin at the site of application, was cleansed free of the test substance using clean swabs of cotton wool soaked in warm water and then dried gently with clean tissue paper. Following each application, there was a rest period of up to 18 hours. Dosing was sequential in group order and performed at approximately the same time each day.

On non-dosing days (except the, day on which functional observations are made) the animals were bandaged (to maintain the procedural routine for the animals) although no dose was given. Control animals were treated in the same manner as test animals except that only bandages were applied.

5. Statistics - Data were evaluated using the SAS (1999) package.

ED_005172C_00001756-00006

C. METHODS:

1. Observations:

1a. Clinical observations

The rats were observed to ensure that they were normal physically and exhibited normal behavior before allocation to the study. Any observations were recorded in the study diary.

Detailed observations were recorded daily prior to dosing and after decontamination for signs of systemic toxicity and skin irritation. Detailed observations were also recorded prior to bandaging and following bandage removal on non-dosing days. Cage-side observations were made as soon as possible after dosing and/or bandaging and on at least one other occasion each day throughout the study.

No detailed observations were recorded on day 22 as the Functional Observation Battery (FOB) was carried out on this day.

1b. Functional observational battery

Detailed clinical assessments (during which each rat was removed from its cage and physically examined for changes in general health status) and quantitative assessments of landing foot splay, muscle weakness (fore- and hindlimb grip strength) and sensory perception (tail-flick test) were made in week 4 for all animals. The observations were made by one observer who was 'blind' with respect to the animal's treatment, and recorded on a computer system by personnel not directly involved in the clinical observations. The presence and/or absence of all listed observations was recorded and the degree of condition noted (slight, moderate or extreme) where appropriate.

The clinical observations included, but were not restricted to, the following list of measures:

a) Assessment of signs of autonomic functions, e.g. lachrymation, salivation, piloerection, exophthalmos, urinary incontinence, diarrhoea, papillary response to light and ptosis.

b) Description, incidence and severity of any convulsions, tremors or abnormal motor movements, both in the home cage and standard arena.

c) Ranking by severity, the subject's reactivity to general stimuli such as removal from the cage or handling.

d) Ranking by severity, the subject's arousal level or state of alertness during observations of the unperturbed subject in the standard (open) arena.

2. Body weight

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The body weight of each rat was recorded daily immediately prior to dosing/bandaging on days 1 to 29 (excepting day 22 when FOB procedures were carried out) and prior to termination on day 29.

3. Food consumption

Food consumption was recorded continuously throughout the study for each rat and calculated, at weekly intervals, as a mean value (g food/rat/day).

4. Ophthalmoscopic examination:

The eyes of all rats were examined before the start of the study. The eyes of rats from the control and high dose groups were examined during the week prior to scheduled termination.

In order to avoid causing undue disturbance to animals currently being dosed on this study the animals for Ophthalmoscopy, FOB and motor activity assessments were moved from room J2.4 to room J2.5 on the day scheduled for these procedures prior to applying the bandages to the remaining replicates in J2.4 in order to minimize the disturbance to the treated animals.

The animals transferred to J2.5 had the FOB carried out prior to Ophthalmoscopy in order to prevent the application of Mydracil to the eye affecting the FOB assessments. Following the completion of Ophthalmoscopy the animals were transferred to the motor activity monitor. Following completion of the assessment of motor activity the animals were returned to J2.5 where they remained until the bandages were removed from all animals in J2.4. The animals in J2.5 were then returned to J2.4.

5. Hematology & Clinical Chemistry:
Blood was collected from all surviving animals at termination. The CHECKED (X) parameters were examined.

a. Hematology

x	Hematocrit (HCT)*	x	Leukocyte differential count*
x	Hemoglobin (HGB)*	x	Mean corpuscular HGB (MCH)*
x	Leukocyte count (WBC)*	x	Mean corpusc. HGB conc.(MCHC)*
x	Erythrocyte count (RBC)*	x	Mean corpusc. volume (MCV)*
x	Platelet count*	x	Reticulocyte count
	Blood clotting measurements*		
x	(Thromboplastin time)		
	(Clotting time)		
x	(Prothrombin time)		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

ED_005172C_00001756-00008

b. Clinical Chemistry

	ELECTROLYTES		OTHER
x	Calcium	x	Albumin*
x	Chloride	x	Creatinine*
	Magnesium	x	Urea nitrogen*
x	Phosphorus	x	Total Cholesterol*
x	Potassium* (K)	x	Globulins
x	Sodium* (NA)	x	Glucose*
	ENZYMES (more than 2 hepatic enzymes, eg., *)	x	Total bilirubin
x	Alkaline phosphatase (AP)*	x	Total protein*
	Cholinesterase (ChE)	x	Triglycerides
x	Creatine phosphokinase	x	Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
x	Alanine aminotransferase (ALT/also SGPT)*		
x	Aspartate aminotransferase (AST/also SGOT)*		
x	Gamma glutamyl transferase (GGT)*		
	Glutamate dehydrogenase		
	Sorbitol dehydrogenase*		

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200

6. Urinalysis*

Urine was collected from all animals. The CHECKED (X) parameters were examined.

x	Appearance*	x	Glucose*
x	Volume*	x	Ketones
x	Specific gravity / osmolality*	x	Bilirubin
x	pH*	x	Blood / blood cells*
x	Sediment (microscopic)		Nitrate
x	Protein*		Urobilinogen

* Optional for 28-day dermal toxicity studies

7. Sacrifice and Pathology

All animals were sacrificed on schedule and subjected to gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs, in addition, were weighed. All submitted tissue from the control and high dose groups, normal skin, skin from the application site and adrenal glands from groups 2 and 3 together with abnormal tissue from all dose groups were trimmed, embedded in paraffin wax and 5um sections cut and stained with hematoxylin and eosin.

ED_005172C_00001756-00009

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
	Tongue	x	Aorta, thoracic*	xx	Brain*+
x	Salivary glands*	xx	Heart*+	x	Peripheral nerve*
x	Esophagus*	x	Bone marrow*	x	Spinal cord (3 levels)*
				x	sciatic nerve
x	Stomach*		Lymph nodes*	x	Pituitary*
x	Duodenum*	xx	Spleen*+	x	Eyes (optic nerve)*
x	Jejunum*	xx	Thymus*+		GLANDULAR
x	Ileum*			xx	Adrenal gland*+
x	Cecum*		UROGENITAL		Lacrimal gland
x	Colon*	xx	Kidneys*+	x	Parathyroid*
x	Rectum*	x	Urinary bladder*	x	Thyroid*
xx	Liver*+	xx	Testes*+		OTHER
	Gall bladder* (not rat)	xx	Epididymides*+	x	Bone (sternum and/or femur)
	Bile duct* (rat)	x	Prostate*		Skeletal muscle
x	Pancreas*	x	Seminal vesicles*	x	Skin* (treated & untreated areas)
	RESPIRATORY	xx	Ovaries*+	x	All gross lesions and masses*
x	Trachea*	xx	Uterus*+		
x	Lung*	x	Mammary gland*		
x	Nose*				
x	Pharynx*				
	Larynx*				

* Recommended for 28-day dermal toxicity studies based on Guideline 870.3200
\+ Organ weights required.

II. RESULTS

There were no changes indicative of systemic toxicity in either sex. Histopathological changes indicative of irritation were seen in skin from the application site in both sexes given 1000 or 300 mg/kg/day and in some males given 30 mg/kg/day,

A. observations:

1. Clinical signs of toxicity -There were no clinical observations indicative of systemic toxicity. A number of observations associated with the skin were seen e.g. desquamation, small scattered scabs and scabs at the edge of the application area. These are consistent with those commonly seen in dermal toxicity studies as a consequence of bandaging, were seen in control animals and at a similar incidence in animals given 30 mg/kg/day. However these findings occurred with increased incidence in animals given 300 or 1000 mg/k,/day and in addition some or all of these animals also had oedema, erythema, staining around the nose, thickening of the skin, and wrinkling .

2. Mortality - There was one mortality. Female 67 (given 300 mg/kg/day) was found dead on day 21 of the study. This animal was in the mid dose group and therefore the death is considered to be unrelated to treatment with Dicamba Tech: (San 837 tech).

ED_005172C_00001756-00010

3. **Neurological Evaluations** (Functional observations battery/Functional clinical observations)-

There were no compound related changes in any of the parameters assessed.

4. **Dermal Irritation** -A number of observations associated with the skin were seen at the application site; desquamation, small scattered scabs These were seen in control animals and at a similar incidence in animals given 30 mg/kg/day. However these findings occurred with increased incidence in animals given 300 or 1000 mg/kg/day and in addition some or all of these animals also had oedema, erythema, staining around the nose, thickening of the skin, and wrinkling .

B. BODY WEIGHT AND WEIGHT GAIN: Body weight in males and females given1000 mg/kg/day and males given 300mg/kg/day were slightly lower than controls but the difference was not consistent or statistically significant. In males there was no clear dose response across the groups.

C. FOOD CONSUMPTION AND EFFICIENCY:

Food consumption - There was no compound-related effect on food consumption.

D. OPHTHALMOSCOPIC EXAMINATION:

The were no abnormalities seen in the eyes of animals given 1000 mg/kg/day.

E. BLOOD ANALYSES:

1. **Hematology** - There were no compound-related effects on the hematological parameters measured. The reduced mean cell hemoglobin in males receiving 1000 mg/kg/day was considered not to be of biological or toxicological significance in the absence of effects in the primary red blood cell parameters (red blood cell count, hemoglobin and mean cell volume). The reduced eosinophil count in males dosed with 300 mg/kg/day was not present at the higher dose and was therefore not considered to be compound-related.

The blood films examined showed no compound-related findings.

2. **Clinical Chemistry** - There were no effects on the clinical chemistry parameters measured, Plasma albumin and plasma cholesterol was slightly lower than controls in males receiving 1000 mg/kg/day but in the absence of any effect in females were considered to be of no toxicological significance. Plasma alkaline phosphatase and alanine aminotransferase activities were lower than controls in all male dose groups. The differences seen were small and are considered to be of no biological significance. Plasma gamma-glutamyl transferase activity at 300 mg/kg/day and plasma creatine kinase activity at 30 mg/kg/day were higher than controls but these differences were not present at the higher doses and were therefore considered not to be compound-related.

ED_005172C_00001756-00011

F. URINALYSIS - There were no compound-related effects on urine clinical chemistry. The male urinary pH was slightly higher than controls at 300 mg/kg/day but this difference was not seen at 1000 mg/kg/day and was considered not to be compound-related, There were no effects on parameters measured in the urinary qualitative tests.

G. Sacrifice and Pathology:

1. Organ weight - No dose-related effects were observed on absolute or relative organ weight.

2. Gross pathology - No compound related abnormalities were observed at sacrifice and gross pathology.

Female #67, found dead on day 21 of the study had a mass on the liver, atrial enlargement and evidence of hemorrhage into the abdominal cavity and thymic lymph nodes; since this was in the mid dose group this death is considered not to be compound-related. Scabs, indicative of treatment induced irritation, were present on the treated area of skin.

In animals surviving to termination, compound-related findings were confined to the skin in the area of application of compound. Scabs were observed in 0, 1, 4 and 7 males and 0,1, 4 and 6 females (at 0, 30, 300 and 1000 mg/kg/day respectively). In the untreated skin scabs were seen more commonly in females but there was no compound-related increase in incidence. These scabs are considered to be associated with the process of bandage application and removal.

3. Microscopic pathology - No compound related abnormalities were observed.

The liver mass in female #67 was caused by infarction of one of the liver lobes. Also present was atrial dilatation, blood filled sinuses in the thymic lymph node, There was evidence of skin irritation i.e. acanthosis/hyperkeratosis, inflammatory cell infiltration, epithelial necrosis and surface exudation. These latter findings are considered to be compound-related.

In animals surviving to termination compound-related findings were confined to treated skin and consisted of acanthosis/hyperkeratosis, inflammatory cell infiltration, epithelial] necrosis, surface debris and occasional epithelial vesicle formation. The majority of animals given 300 and 1000 mg/kg/day showed these changes but they were more severe in the 1000 mg/k/day group, Minor changes consisting of mainly acanthosis/hyperkeratosis were observed in some males given 30 mg/kg/day of compound.

A lesion in the adrenal i.e. necrosis/fibrosis/ vacuolation/pigmentation, was seen in 3 males given 1000 mg/kg/day of compound. However, a similar reaction was also seen in 1 male given 300 mg/kg and one female given 30 mg/kg/day and one- control female. It is considered not to be compound-related..

ED_005172C_00001756-00012

III. DISCUSSION and CONCLUSIONS

A. INVESTIGATORS' CONCLUSIONS:

Dermal administration of 1000 mg Dicamba tech. (SAN 837 tech.)/kg/day for 21 days in a 28 day period to male and female rats produced no evidence of systemic toxicity. There were histopathological changes in skin at the application site indicative of skin irritation. Similar effects were seen in animals given 300 mg/kg/day although the changes associated with irritation were less severe, Males given 30 mg/kg/day showed minimal histopathological signs of skin irritation in a few animals. There were no effects in females at this dose level. The NOAEL for systemic toxicity in this study is considered to be 1000 mg/kg/day.

B. REVIEWER COMMENTS: This study is an extremely detailed evaluation of the potential for toxicity during a 28-day repeated dermal dosing study with dimethoate technical. This reviewer is unable to suggest or recommend any additional observations/testing that could contribute to the value of the study. Evidence of systemic toxicity was not observed at 1000 mg/kg/day, the highest dose tested. Therefore, a LOAEL was not determined. The NOAEL is 1000 mg/kg/day, the highest dose tested. Histopathological changes indicative of irritation were seen in skin from the application site in both sexes given 1000 or 300 mg/kg/day and in some males given 30 mg/kg/day.

C. STUDY DEFICIENCIES: none

ED_005172C_00001756-00013

DATA FOR ENTRY INTO ISIS

Subchronic Dermal (28 day) Study - rodents (870.3200)

PC code	MRID	Study	Species	Duration	Route	Admin	Dose range mg/kg/day	Doses mg/kg/day	NOAEL mg/kg/day	LOAEL mg/kg/day	Target organ	Comments
029801	45814501	subchronic	rat	28 days	dermal	dermal	30-1000	30, 300, 1000	1000 (HDT)	not determined	none	no Systemic toxicity observed. dermal irritation observed at application site

ED_005172C_00001756-00014

DATA EVALUATION RECORD

DICAMBA

Study Type: 82-1a; 13-Week Oral Toxicity Study in Rats

Work Assignment No. 1-1-1B (MRID 44623101)

Prepared for
Health Effects Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1921 Jefferson Davis Highway
Arlington, VA 22202

Prepared by
Pesticides Health Effects Group
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Joan L. Harlin, M.S.

Signature: Joan L. Harlin
Date: 4/14/99

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Signature: Kathleen P. Ferguson
Date: 4/14/99

Program Manager
Mary L. Menetrez, Ph.D.

Signature: Mary L Menetrez
Date: 5/24/99

Quality Assurance
Steven Brecher, Ph.D.

Signature: Steven Brecher
Date: 5/21/99

Disclaimer

This Data Evaluation Record may have been altered by the Health Effects Division subsequent to signing by Dynamac Corporation personnel.

ED_005172C_00001756-00015

EPA Reviewer: Yung G. Yang, Ph.D. Signature: Yung G Yang
Toxicology Branch, Health Effects Division (7509C) Date: 8/24/2005
Work Assignment Manager: PV Shah, Ph.D. Signature: PV Shah
Health Effects Division (7509C) Date: 8/24/05

TXR: 0052528

DATA EVALUATION RECORD

STUDY TYPE: 90-Day subchronic toxicity [feeding] - rat; OPPTS 870.3100; OECD 408

P.C. CODE: 029801 DP BARCODE: D249152
SUBMISSION CODE: S548172 OPPTS Number: 870.3100

TEST MATERIAL (PURITY): Dicamba TC (89.4% a.i.)

SYNONYMS: 3,6-Dichloro-2-methoxybenzoic acid

CITATION: Doubovetzky, M. (1997) Dicamba TC: 13-Week feeding study in rats (including 4-week recovery). Novartis Crop Protection AG, Department of Toxicology, B.881, CH-4132 Muttenz 1/Switzerland. Study No. 602R. Report No. 97/059. April 25, 1997. MRID 44623101. Unpublished.

SPONSOR: Novartis Crop Protection AG, Department of Toxicology, B.881, CH-4132 Muttenz 1/Switzerland

EXECUTIVE SUMMARY: In a 13-week subchronic toxicity study (MRID 44623101), dicamba technical (89.4% a.i.) was administered to HanIbm:WIST (Wistar) rats (10 or 20 rats/sex/dose) by feeding at dose levels of 0, 500, 3000, 6000, or 12,000 ppm (equivalent to 0/0, 40.1/43.2, 238.7/266.4, 479.4/535.6, or 1000.0/1065.3 mg/kg/day [M/F]) for 13 weeks. Following 13 weeks of treatment, 10 rats/sex/dose were sacrificed. Rats (10/sex) in the control and 12,000 ppm groups were maintained for a 4-week recovery period to determine the reversibility of effects.

No treatment-related deaths were observed in any treatment group. The liver was the target organ, as evidenced by microscopic liver changes associated with clinical serum chemistry changes and increased relative (to body) liver weights (↑20-23%) in both sexes at the high dose. The livers of the 12,000 ppm females exhibited slight centrolobular hepatocyte hypertrophy (4/10) and an increased incidence of minimal to moderate hepatocellular pigmentation (5/10). Both sexes exhibited increased alkaline phosphatase (↑62-76%), serum alanine aminotransferase (↑59-66%), and serum aspartate aminotransferase (↑29%) activities compared to the controls. Females exhibited an increase in mean gamma glutamyl transferase activity (↑136%) while males showed a decrease activity (↓50%) compared to the controls.

Other effects observed in the 12,000 ppm rats were transient hypothermia (weeks 1-4), reduced activity, slower movements, decreased food consumption, and less efficient food utilization than

ED_005172C_00001756-00016

the controls throughout the treatment period. Lower mean final body weights (↓18-20%), body weight gains (↓28-40%) and adipose tissue content were observed compared to the controls. Decreases in protein (↓10-15%) and globulin (↓16-26%) levels were observed in both sexes. In females, decreased mean hemoglobin concentration (↓4%) and red blood cell counts (↓4%), and decreased mean corpuscular hemoglobin concentration (↓3%) were observed. Significant ($p<0.05$ or $p<0.01$) increases of white blood cell count (↑13%) and lymphocyte count (↑33%) were observed in 12000 ppm females compared to the controls. Males had a lower mean platelet count (↓7%) and shorter partial thromboplastin time (↓11%) compared to the controls. Urinalysis showed that males excreted more triple phosphate crystals in the 12000 ppm group, whereas females excreted more uric acid crystals in the 12000 and 6000 ppm groups at week 12. Following a 4-week recovery period, all observed effects were recovered.

The LOAEL for this study is 12,000 ppm (1000 mg/kg/day), based on clinical signs, reduced body weight gains, hematological and clinical serum chemistry changes in both sexes, centrolobular hepatocyte hypertrophy and hepatocellular pigmentation in females, and increased relative (to body) liver weights for both sexes. The NOAEL is 6000 ppm (479 mg/kg/day).

This 13-week subchronic toxicity study is classified **acceptable/guideline (870.3100)** and satisfies the guideline requirement for a subchronic toxicity study in rodents.

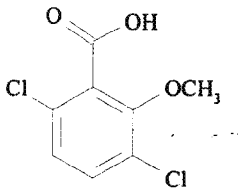
COMPLIANCE: Signed and dated GLP, Quality Assurance, Data Confidentiality, and Flagging statements were provided.

ED_005172C_00001756-00017

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test material: Dicamba TC
   Description: White powder
   Lot #: 52504710
   Purity: 89.4% (w/w) a.i.
   Stability of compound: Expiration date reported to be July 21, 1999
   CAS #: 1918-00-9
   Structure:



2. Vehicle: Diet

3. Test animals: Species: Rat
   Strain: HanIbm: WIST (Wistar)
   Age and weight at study initiation: Approximately 6 weeks old; body weight at Week 0, males - 122-161 g; females - 108-134 g
   Source: BRL Breeding Laboratories, CH-4414 Füllinsdorf, Switzerland
   Housing: Three or four animals per Macrolon size 4 plastic cages with solid bottoms
   Diet: KLIBA powdered diet No. 343 (Klingentalmühle AG Basel, CH-4303 Kaiseraugst, Switzerland), ad libitum, offered fresh weekly
   Water: Tap water, ad libitum, offered fresh weekly
   Environmental conditions:
   Temperature: 23 ± 2° C
   Humidity: 55 ± 25%
   Air Changes: 6-10/hour
   Photoperiod: 12 Hours light/12 hours dark
   Acclimation period: 14 Days

B. STUDY DESIGN:

1. In life dates - Treatment start: 10/31/96 End: 3/4/97
   Recovery phase start: 3/4/96 End: 4/4/97

2. Animal assignment - Rats were assigned to the test groups in Table 1 immediately after receipt using a computer-generated cage distribution plan, in chronological order of unpacking. At the start of the study, the body weight of each animal was within 20% of the mean value for each sex.

ED_005172C_00001756-00018

Table 1. Study design.[a]

Test Group	Dose (ppm)	Dose (mg/kg/day)		Animals # in toxicity phase		Animals # in recovery phase	
		Male	Female	Male	Female	Male	Female
K Control	0	0	0	10	10	10	10
A Low	500	40.1	43.2	10	10	--	--
B Mid	3000	238.7	266.4	10	10	--	--
C Mid High	6000	479.4	535.6	10	10	--	--
D High	12,000	1000	1065.3	10	10	10	10

a Doses were selected based on results of a previous toxicity study in rats (no further information provided).

3. Treatment preparation and dosing - Four separate batches of each test diet were prepared for weeks 0-3, 4-7, 8-11, and 12-end and stored at room temperature until use. A premix was prepared by adding dicamba to a portion of powdered diet and diluting with untreated diet to yield the desired concentration of dicamba. Subsamples of the 0-week (0 day) feed preparations were collected from the top, middle, and bottom of the mixing container for homogeneity and stability analyses. Subsamples of the feed preparations were collected at 0 days and 4, 8, and 12 weeks for concentration analyses.

   Results:
   Homogeneity analyses:
   500 ppm: 95.6-101.6% of nominal (mean 98.1%)
   3000 ppm: 96.8-101.7% of nominal (mean 98.7%)
   6000 ppm: 95.6-108.3% of nominal (mean 101.9%)
   12,000 ppm: 101.4-101.9% of nominal (mean 101.7%)

   Stability analyses: All dose levels were stable when stored at room temperature for up to 36 days (99.5-101.3%).

   Concentration analyses (week 4, 8, and 12):
   500 ppm: 90.8/91.4/91.2% (mean 92.9%)
   3000 ppm: 97.7/95.6/98.7% (mean 97.7%)
   6000 ppm: 104.7/98.5/92.4% (mean 98.2%)
   12,000 ppm: 107.6/95.7/94.6% (mean 99.1%)

   The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

4. Statistics - Analyses of parametric data were performed using standard one-way ANOVA

ED_005172C_00001756-00019

followed by Dunnett's test for equal variances. Analyses of nonparametric data were performed using the Kruskal-Wallis test followed by Mann Whitney-U. Count data were analyzed using Chi-square followed by Fisher's exact test.

C. METHODS:

1. Observations - Animals were observed twice daily on weekdays and once daily on weekends and holidays for mortality and signs of ill-health. All animals received a weekly detailed examination that included palpation.

2. Body weight - All animals were weighed weekly, beginning two weeks prior to treatment and then throughout study termination. The animals were weighed on the first day of each week.

3. Food consumption, feed efficiency, and compound intake - Food consumption (g) for each cage (3 or 4 rats/cage) was determined weekly during the study by calculating the difference in food given and food remaining in the hopper at the end of the week divided by the total number of animals. Feed efficiency was determined during the study by calculating the mean food conversion ratios (total mean food consumption/mean body weight gain). Test substance intake was calculated for each cage using the mean body weight and food consumption data, and the nominal dietary test material concentration.

4. Ophthalmoscopic examination - Ophthalmoscopic examinations were conducted on all test animals prior to treatment (week -1) and on Group K (control) and Group D (12,000 ppm; recovery) animals only during week 12. Due to suspected ocular changes in females during the week 12 examination, the examinations were extended to include all females in the intermediate dose group, and a supplementary examination was performed during week 17 in the female recovery groups. The examinations were performed using an indirect ophthalmoscope following administration of a mydriatic.

5. Blood - For hematology, blood was collected from all animals in the main subgroups during week 12 and from all animals in the recovery subgroups during week 17. The rats were placed in partly darkened metabolism cages and deprived of food but not water. Following urine collection, blood was drawn from each animal by venipuncture of the sublingual vein under Isofluran anaesthesia. The CHECKED (X) hematology and clinical chemistry parameters were examined.

ED_005172C_00001756-00020

a. Hematology

X	Hematocrit (HCT)*	X	Mean corpuscular HGB (MCH)
X	Hemoglobin (HGB)*	X	Mean corpusc. HGB conc.(MCHC)
X	Leukocyte count (WBC)*	X	Mean corpusc. volume (MCV)
X	Erythrocyte count (RBC)*	X	Leukocyte differential count*
X	Platelet count*	X	Neutrophil count
	Blood clotting measurements*	X	Lymphocyte count (% and Th/cmm)
	(Clotting time)	X	Monocyte count
X	(Prothrombin time)	X	Eosinophil count
X	(Partial thromboplastin time)	X	Basophil count
		X	Red blood cell morphology[a]

* Required for subchronic toxicity studies
a Red blood cell morphology investigations were performed on control and high dose animals only.

b. Clinical Chemistry

	ELECTROLYTES		OTHER
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium	X	Blood urea nitrogen*
X	Phosphorus*	X	Cholesterol
X	Potassium*	X	Globulin
X	Sodium*	X	Glucose* (fasting)
		X	Total bilirubin
	ENZYMES	X	Total serum protein (TP)*
		X	Triglycerides
X	Alkaline phosphatase		A/G Ratio
	Cholinesterase (ChE)		
X	Creatine phosphokinase		
X	Lactic acid dehydrogenase (LDH)		
X	Serum alanine aminotransferase		
X	Serum aspartate aminotransferase		
X	Gamma glutamyl transferase (GGT)		

* Required for subchronic toxicity studies.

7. Urinalysis - Urine was collected at the end of the treatment and recovery periods. Animals were housed in partially darkened metabolism cages and deprived of food but not water. Urine was collected overnight on ice. The CHECKED (X) parameters were examined in all samples analyzed.

ED_005172C_00001756-00021

X	Appearance	X	Red blood cells (RBC)
X	Color	X	White blood cells (WBC)
X	Volume	X	Epithelial cells
X	Specific gravity	X	Calcium oxalate crystals
X	pH	X	Triple phosphate crystals
X	Protein	X	Amorphousurate
X	Glucose	X	Uric acid crystals
X	Ketones	X	Casts
X	Occult Blood	X	Parasites
X	Urobilinogen	X	Parasites
X	Bilirubin	X	Bacteria
X	Nitrite		

* Required for subchronic toxicity studies.

8. Sacrifice and pathology - All surviving animals in the main subgroups were sacrificed after 13 weeks of treatment by CO_2 inhalation. All animals in the recovery subgroups were sacrificed by CO_2 inhalation after a 4-week recovery period following 13 weeks of treatment. All sacrificed animals in the main subgroups and recovery subgroups were subjected to a complete gross pathological examination. The CHECKED (X) tissues were collected for histological examination. The (XX) organs were weighed.

	DIGESTIVE SYSTEM		CARDIOVASC./HEMAT.		NEUROLOGIC
X	Tongue	X	Aorta*	XX	Brain*
X	Salivary glands*	XX	Heart*	X	Spinal cord (3 levels)
X	Esophagus*	X	Bone marrow*	X	Pituitary*
X	Stomach*	X	Lymph nodes*	X	Eyes (optic n.)*
X	Duodenum*	XX	Spleen*		
X	Jejunum*	X	Thymus*		
X	Ileum*				GLANDULAR
X	Cecum*				
X	Colon*		UROGENITAL		
X	Rectum*			XX	Adrenal gland*
XX	Liver*	XX	Kidneys*	X	Lacrimal gland
	Gall bladder	X	Urinary bladder*	X	Mammary gland
X	Pancreas*	XX	Testes*	X	Thyroids*
		X	Epididymides	X	Parathyroids*
		X	Prostate		
	RESPIRATORY	X	Seminal vescicles		OTHER
		XX	Ovaries*		
X	Trachea*	X	Uterus* (and cervix)	X	Bone* (femur, stiffle joint, sternum)
X	Lung*	X	Vagina		
	Pharynx			X	Skeletal muscle*
	Larynx			X	Skin*
				X	All gross lesions and masses*
				X	Skull and ears

* Required for subchronic toxicity studies.

ED_005172C_00001756-00022

II. RESULTS

A Observations

1. Mortality - No animals died prematurely.

2. Clinical signs - The 12,000 ppm male and females exhibited slowed movements and reduced activity and were cold to the touch. The incidence of slowed movements was higher during weeks 0-12 (9-20 males; 16-20 females) than week 13 (5 males; 8 females). Reduced activity was most prevalent in males during weeks 0-3 (8-14 rats), then decreased during weeks 4-6 (5-6 rats) and 7-12 (1-2 rats), whereas most females (15-20) exhibited reduced activity during weeks 0-12. Both sexes were cold to the touch during weeks 1-4 (8-19 males; 15-20 females). The behavior and appearance of rats in the 6000, 3000, and 500 ppm groups were similar to the control rats. Sporadic incidences of dermal crusted areas, fur loss, erythema or kinked/shortened tail were observed in all treatment and control groups.

B. Body weight and weight gain - For the 12,000 ppm group, mean body weights and body weight gains of males were 18 and 28% lower, respectively, and of females were 20 and 40% lower, respectively, than the controls by the end of the 13-week treatment period. Both sexes gained significantly ($p<0.01$) more weight than the controls during a 4-week recovery period, although the final body weight gain was 8% lower for males and 11% lower for females compared to the control gains. Body weights and body weight gains of rats in the 6000, 3000, and 500 ppm groups were similar or greater than the controls throughout the study (Table 2).

ED_005172C_00001756-00023

Table 2. Mean body weight and body weight gains (g) of rats before and during treatment with dicamba.[a]

Treatment rate (ppm)	Body weight (g)				13-Week body weight gain	
	0 Weeks	4 Weeks	13 Weeks	17 Weeks	Total (g)	% of Control gain
Males						
0	145.2	298.9	412.7	444.5	267.5	---
500	145.7	291.7	417.2	---[b]	271.5	+1
3000	143.7	303.5	429.9	---	286.2	+7
6000	147.1	309.3	427.2	---	280.1	+5
12,000	146.8	241.4*	339.7*	411.8	192.9	-28
Females						
0	120.4	187.1	236.8	259.4	116.4	---
500	118.9	185.6	234.6	---	115.7	-1
3000	121.9	193.3	232.8	---	110.9	-5
6000	121.7	194.1	238.9	---	117.2	+1
12,000	121.2	156.7*	190.6*	231.0*	69.4	-40

a Body weights obtained from Table 3, pages 47-50 of the study report. Body weight gains were calculated by the reviewers.
b Not applicable
* Significantly different from the control, $p<0.01$.

C. Food consumption and compound intake

1 Food consumption - Food consumption (g/animal/day) by the 12,000 ppm males was less than the control group throughout the study, with the most pronounced differences ($p<0.05$ or 0.01) during week 1 (↓43%) and weeks 2-5 (↓13-18%). The 12,000 ppm males consumed ≤8% less food than the controls during the remaining treatment weeks, except during week 9 (↓14%, $p<0.01$). Overall food consumption by the 12,000 ppm males was 13% lower ($p<0.01$) than the controls (Table 3). During a 4-week recovery period, food consumption by the 12,000 ppm males was equal to the controls.

Food consumption by the 12,000 ppm females was also less than the control group throughout the study, with the most pronounced differences during week 1 (↓40%) and weeks 2-5 (↓19-23%). The 12,000 ppm females consumed 4-13% less food than the controls during the remaining treatment weeks. Overall food consumption by the 12,000 ppm males was 13% lower ($p<0.01$) than the controls (Table 3). During a 4-week recovery period, food consumption by the 12,000 ppm females was 11% higher than the controls.

ED_005172C_00001756-00024

Mean weekly food consumption by rats in the 6000, 3000, and 500 ppm groups was, in general, similar to or higher than the controls throughout the study. Feed efficiency for all treatment groups was similar to the controls.

Table 3. Mean food consumption (g) by rats before and during treatment with dicamba.[a]

Treatment rate (ppm)	Body weight (g)				
	1 Week	3 Weeks	5 Weeks	13 Weeks	17 Weeks
Males					
0	22.1	25.5	25.3	20.0	22.4
500	21.4	24.2	25.1	20.4	---[b]
3000	21.2	24.9	25.6	20.4	---
6000	20.8	25.4	26.7	21.1	---
12,000	12.7*	20.9*	22.0*	18.8	22.8
Females					
0	16.6	17.8	18.1	13.7	16.8
500	15.9	17.0	17.5	13.6	---
3000	15.9	17.9	18.7	13.3	---
6000	14.9	16.7	17.7	15.1	---
12,000	9.9*	14.4*	14.0*	13.2	17.1

a Data obtained from Table 4, pages 51-54 of the study report.
b Not applicable
* Significantly different from the control, $p<0.01$.

2. Compound intake - Actual compound intake by rats in the 12,000, 6000, 3000, and 500 ppm groups was presented in Table 1.

3. Feed efficiency - Feed efficiency for the 12,000 ppm males was reduced compared to the controls during the 13-week treatment period, based on a higher food conversion ratio compared to the controls (9.7 vs. 8.0). During a 4-week recovery period, the 12,000 ppm males showed a more efficient utilization of food, based on a much lower food conversion ratio compared to the controls (10.1 vs. 18.4). Feed efficiency for the 12,000 ppm females was also reduced compared to the controls during the 13-week treatment period, based on a higher food conversion ratio compared to the controls (18.8 vs. 13.0). During a 4-week recovery period, the 12,000 ppm females showed a more efficient utilization of food, based on a much lower food conversion ratio compared to the controls (15.1 vs. 25.4). Feed efficiency for the 6000, 3000, and 500 ppm treatment groups was similar to the controls throughout the 13-week treatment period.

ED_005172C_00001756-00025

D. Ophthalmoscopic examination - Females in the 12,000 ppm group exhibited a higher incidence of thin retinal blood vessels (8/20 vs. 2/20 controls). No differences were observed between the 12,000 ppm males and 6000, 3000, and 500 ppm male and female groups compared to the controls.

E. Blood work

a. Hematology - Males in the 12,000 ppm group had a 7% lower mean platelet count ($p<0.01$) and a 11% shorter partial thromboplastin time ($p<0.01$) compared to the controls (Table 4). Females in the 12,000 ppm group had mean hemoglobin concentration and red blood cell count values that were each 4% lower ($p<0.05$ or 0.01), and a 3% lower mean corpuscular hemoglobin concentration ($p<0.01$) compared to the controls. The 12,000 ppm females also exhibited mean white blood cell and lymphocyte counts 13 and 33% higher, respectively, than the control counts ($p<0.05$). Following a 4-week recovery period, hematology parameters for the 12,000 ppm male and females were similar to the corresponding control values. Other differences observed in the treatment groups were neither toxicologically significant nor concentration-dependent, and were considered incidental in nature.

Table 4. Selected hematology parameters in rats following 12 weeks of treatment with dicamba.[a]

Treatment rate (ppm)	Hemoglobin (g/dL)	RBC[b] (10^6/mm3)	MCHC[b] (g/dL)	Platelet count (10^6/mm3)	WBC[b] (10^6/mm3)	PTT[b] (sec)	Lymph[b] (10^6/mm3)
Males							
0	16.54	9.473	35.67	734.1	5.760	20.1	4.379
500	16.44	9.316	35.14*	673.8	5.505	19.7	4.307
3000	16.43	9.324	35.44	697.1	5.453	20.2	3.972
6000	16.25	9.218	35.26*	681.9	5.303	20.6	4.216
12,000	16.60	9.363	35.57	653.5**	6.168	18.7**	4.958
Females							
0	15.91	8.476	35.31	681.5	3.701	19.9	3.013
500	15.78	8.530	35.38	669.5	3.736	21.3	2.825
3000	15.76	8.525	35.20	711.7	3.197	20.2	2.353
6000	15.64	8.469	34.85*	699.5	3.844	20.0	3.059
12,000	15.31**	8.164*	34.84**	596.9**	4.750*	18.7**	3.997*

a Data obtained from Table 6, pages 59-76 of the study report.
b RBC - red blood cell count; MCHC - mean corpuscular hemoglobin concentration; WBC- white blood cell count; PTT - partial thromboplastin time; Lymph - lymphocytes.
* or ** Significantly different from the control at $p<0.05$ or 0.01.

ED_005172C_00001756-00026

b. Clinical chemistry - Both sexes in the 12,000 ppm group exhibited significant ($p<0.01$) decreases in protein (males, 15%; females, 10%) and globulin (males, 26%; females, 16%), and increases in alkaline phosphatase (males, 62%; females, 76%), serum alanine aminotransferase (males 59%; females, 66%), and serum aspartate aminotransferase (both sexes, 29%) activities compared to the controls (Table 5). The 12,000 ppm females also exhibited an increased mean gamma glutamyl transferase activity that was 136% higher ($p<0.01$) than the control mean but males showed a decrease of activity. Other significant ($p<0.05$) observed effects in the 12,000 ppm groups were decreased triglyceride (48%), cholesterol (26%), and glucose (14%) levels in males, and increased triglycerides (62%), cholesterol (32%), creatinine (15%), and phosphorus (20%) levels in females. Following a 4-week recovery period, clinical blood chemistry parameters for the 12,000 ppm animals were similar to the corresponding control values.

Table 5. Selected clinical chemistry parameters in rats following 12 weeks of treatment with dicamba.[a]

Dose Group (ppm)	Protein (g/L)	Glob[b] (g/L)	ALK[b] (IU/L)	ALT[b] (IU/L)	AST[b] (IU/L)	GGT[b] (IU/L)	Tri[b] (mmol/L)	Chol[b] (mmol/L)	Creat[b] (µmol/L)	Glu[b] (mmol/L)	Phos[b] (mmol/L)	Urea (mmol/L)
Males												
0	74.70	37.29	70.0	16.40	67.30	5.733	0.813	1.070	51.47	7.147	1.795	6.838
500	73.66	36.73	64.6	13.80	67.70	3.579	0.561	0.926	53.01	7.511	1.786	6.918
3000	78.67	39.76	75.4	24.60	79.50	4.508	0.807	1.118	58.67	6.999	1.881	7.142
6000	72.33	34.75	73.4	21.20*	72.40	3.300	0.588	0.961	58.32	6.704	1.852	7.729
12,000	63.42**	27.47**	113.3**	26.10**	86.75**	2.894*	0.426**	0.795**	58.24	6.174**	1.924	8.181*
Females												
0	75.98	34.53	28.8	14.80	66.25	3.194	0.426	0.822	61.08	6.459	1.394	7.881
500	77.17	35.01	29.1	18.60	78.90	2.735	0.407	0.937	62.08	6.889	1.316	8.300
3000	79.75	36.99*	26.9	19.20	69.90	2.420	0.401	0.901	65.09	6.775	1.515	7.914
6000	78.18	35.66	37.7	21.80	72.30	2.457	0.457	0.872	70.40	6.668	1.555	8.242
12,000	68.59**	29.01**	50.6**	24.50**	85.68**	7.548*	0.691**	1.082**	70.54**	6.389	1.674**	9.009

a Data obtained from Table 7, pages 77-87 of the study report.
b ALP - alkaline phosphatase; ALT - serum alanine aminotransferase; AST - serum asparatate aminotransferase; GGT - gamma glutamyl transferase; Tri - triglycerides; Chol - cholesterol; Creat - creatinine; Glu - glucose; Phos - phosphorus.
* Significantly different from the control, $p<0.05$.
** Significantly different from the control, $p<0.01$.

F. Urinalysis - Males in the 12,000 ppm group had a higher incidence of urine containing triple phosphate crystals (15/20 positive samples vs. 7/20 in controls) after 12 weeks of treatment. Females in the 12,000 and 6000 ppm groups had a higher incidence of urine containing uric acid crystals (12/20 in 12,000 ppm group and 6/10 in 6000 ppm group vs. 1/20 in controls) after 12 weeks of treatment. Following a 4-week recovery period, no crystals were found in urine from the 12,000 ppm males and 12,000 and 6000 ppm females. For all treatment groups, other differences in urine parameters were not clearly dose-related and were

ED_005172C_00001756-00027

attributed to normal biological variation.

G. Sacrifice and pathology

1. Organ weight - Animals in the 12,000 ppm group had higher mean relative liver (to body) weights in males and females (23 and 20%, respectively) ($p<0.01$), than the controls (Table 6). Following a 4-week recovery period, the relative (to body) liver weights of both sexes were similar to the controls. No other differences in absolute or relative organ weights were observed between the treated and control groups.

Table 6. Absolute and relative (to body) liver weights in rats following 13 weeks of treatment with dicamba.[a]

Treatment rate (ppm)	Liver	
	Absolute (g)	Relative (to body)
Males		
0	14.19	3.43
500	14.38	3.42
3000	15.17	3.52
6000	15.29	3.56
12,000	14.01	4.22* (↑23%)
Females		
0	8.26	3.52
500	8.68	3.65
3000	8.56	3.60
6000	8.58	3.59
12,000	7.89	4.24* (↑20%)

a Data obtained from Table 9, pages 104-115 of the study report.
* Significantly different from the control, $p<0.01$.

2. Gross pathology - Both sexes in the 12,000 ppm group exhibited a reduction of adipose tissue (1/10 males; 6/10 females) that correlated with a decreased mean final body weight. Following a 4-week recovery period, the adipose tissue did not differ from the controls. No treatment-related gross abnormalities were observed in the other treatment groups.

3. Microscopic pathology

a) Non-neoplastic - The 12,000 ppm females exhibited minimal to slight centrolobular

ED_005172C_00001756-00028

hepatocyte hypertrophy (4/10, $p<0.01$) and an increased incidence of minimal to moderate hepatocellular pigmentation (5/10, $p<0.001$) that correlated with an increased relative (to body) liver weight. Following a 4-week recovery period, the livers of the 12,000 ppm females were histopathologically similar to the control livers. No other test group exhibited hepatocellular changes following treatment, except the liver of one 3000 ppm female that showed minimal hepatocytic pigmentation that was considered to be toxicologically insignificant. No other differences in microscopic alterations were observed in any treatment group. Although the 12,000 ppm animals exhibited a marginal increase in the mean severity of foamy macrophage foci of the lung compared to the controls, the lesions were slight, toxicologically insignificant, within the expected range for rats of this age and strain, and statistically insignificant, and, therefore, not related to treatment. No microscopic abnormalities related to treatment were observed in any other treatment group.

b) Neoplastic - No neoplastic tissue was observed in rats from any test group.

III. DISCUSSION

A. Investigator's conclusions - The study author concluded that treatment-related effects occurred in the 12,000 ppm rats and included reductions in body weight gain and food intake, neurobehavioral signs, significant changes in hematology and clinical blood chemistry parameters, and increased relative (to body) liver weights. The 12,000 ppm females also exhibited slight centrolobular hepatocyte hypertrophy and an increased incidence of minimal to moderate hepatocellular pigmentation. Following a 4-week recovery period, these effects were partially reversed. No treatment-related effects were observed in the other treatment groups. Based on these findings, the study author concluded that the NOAEL is 6000 ppm (479 mg/kg/day for males; 536 mg/kg/day for females).

B. Reviewer's discussion - We agree with the study author's conclusion that the LOAEL for this study is 12,000 ppm and the NOAEL is 6000 ppm. No treatment-related deaths were observed in any treatment group. The liver was the target organ, as evidenced by microscopic liver changes associated with clinical blood chemistry changes and increased relative (to body) liver weights (20-23%) in both sexes. The livers of the 12,000 ppm females exhibited slight centrolobular hepatocyte hypertrophy (4/10) and an increased incidence of minimal to moderate hepatocellular pigmentation (5/10). Other changes that indicated liver damage in both sexes were increased alkaline phosphatase (62-76%), serum alanine aminotransferase (59-66%), and serum aspartate aminotransferase (both sexes, 29%) activities compared to the controls. Females exhibited increased gamma glutamyl transferase activity (↑136%) compared to the controls but the male showed a decrease in GGT activity (↓50%).

The 12,000 ppm rats exhibited a transient hypothermia (weeks 1-4) and were less active and had slower movements than the controls for the entire treatment period. Body weights, body weight gains, food consumption, and feed efficiencies were reduced in both sexes. Males had 18% lower mean body weights and 28% lower mean body weight gains compared to the controls at the end of the treatment period, corresponding decreases in females were 20 and

ED_005172C_00001756-00029

40%. Reduced adipose tissue in both sexes (1/10 males; 6/10 females) correlated with decreased mean final body weights. Although males and females gained significantly ($p<0.01$) more weight than the controls during a 4-week recovery period, final body weight gains were 8-11% lower than the control gains. Both sexes had decreased protein (10-15%) and globulin (16-26%) levels that may have been related to reduced food intake throughout the treatment period. Females had decreased mean hemoglobin concentration and red blood cell counts (each 4%), and decreased mean corpuscular hemoglobin concentration (3%), increased white blood cell count (13%), and lymphocyte count (33%); these differences were significant ($p<0.05$ or $p<0.01$) compared to the control values. Males had a lower mean platelet count (7%) and shorter partial thromboplastin time (11%) compared to the controls. Urinalysis showed males excreted more triple phosphate crystals and females excreted more uric acid crystals at week 12. The 12,000 ppm females had a higher incidence of thin retinal blood vessels that may have been treatment-related. By the end of a 4-week recovery period, all effects observed in the 12,000 ppm rats were recovered.

The LOAEL for this study is 12,000 ppm (1000 mg/kg/day), based on clinical signs, reduced body weight gains, hematological and clinical blood chemistry changes in both sexes, centrolobular hepatocyte hypertrophy and hepatocellular pigmentation in females, and increased relative (to body) liver weights for both sexes. The NOAEL is 6000 ppm (479 mg/kg/day).

This 13-week subchronic toxicity study is classified **acceptable/guideline (870.3100)** and satisfies the guideline requirement for a subchronic toxicity study in rodents.

IV. STUDY DEFICIENCIES

No significant deficiencies were noted for this study.

ED_005172C_00001756-00030